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Claim 19, last line, change "intact, native human papillomavirus virions" to --intact, infectious human papillomavirus virions--.

Claim 50, last line, change "intact, native human papillomavirus virions" to --intact, infectious human papillomavirus virions--.

Claim 63, line 4, change "intact, mature human papillomavirus viruses" to --an intact, infectious human papillomavirus virions--.

REMARKS

Entry of the foregoing amendments, reconsideration and reexamination of the subject application, as amended, pursuant to and consistent with 37 C.F.R. §1.112, and in light of the remarks which follow, are respectfully requested.

By the present amendments, Claims 1, 12, 19, 50 and 63 have been amended to expedite prosecution. In particular, the phrase "intact, native human papillomavirus virions" has been changed to "intact, infectious human papillomavirus virions". Also, in Claim 63, the phrase "intact, mature human papillomavirus virions" has been changed to "intact, infectious human papillomavirus virions". Upon entry of the present amendments, Claims 1-3, 10-19, 21-26, 46, 47, 50 and 63 will remain pending. Support for the amendatory claim language may be found, e.g., at page 10, lines 1-12 of the as-filed application.

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Turning now to the Office Action, the Examiner's withdrawal of numerous of the previous prior art rejections is acknowledged. The only remaining prior art rejections from the previous Office Action is that of Claims 1-3, 10, 12, 15, and 59-62 based on Carter et al (*Virology*, 182, 513-521 (1991)), and that of Claims 16, 23, 24, 52 and 55 to 68 based on Carter et al in view of Danos et al.

The Office Action maintains that these claims are anticipated, or in the alternative, rendered obvious by Carter et al, alone or in combination with Danos et al.

This rejection is only applied against the claims which embrace HPV-1 L1 proteins which reproduce the antigenicity and exhibit the same conformation as intact, native (now infectious) human papillomavirus virions. Essentially, the Office Action indicates that it is reasonable to conclude that the HPV-1 L1 proteins obtained by Carter et al, which were expressed in *Saccharomyces cerevisiae* would "inherently" mimic the conformation of an L1 protein expressed on the surface of intact native HPV-1 virions. The Office Action substantiates this position by reliance on the fact that these proteins are disclosed by Carter et al to possess the expected molecular weight of native, intact HPV-1 L1 protein and also based on the fact that these proteins apparently bind to monoclonal antibodies specific to HPV-1 L1 protein.

However, Applicants respectfully submit that neither of these facts substantiates a reasonable conclusion that the HPV-1 L1 protein obtained by Carter et al exhibits "appropriate confor-

mation". At the outset, Applicants advise that by "appropriate conformation", Applicants refer to an L1 protein which exhibits the same conformation as an L1 protein expressed by intact, native (infectious) HPV virions, e.g., HPV-1 virions. Thus, the HPV-1 L1 protein of Carter et al must contain the same conformational epitopes as an HPV-1 L1 protein expressed on the surface of intact infectious HPV-1 virions to have "appropriate conformation". Based on the following, the anticipatory rejection must be withdrawn because Carter et al contains no evidence which would allow one skilled in the art to conclude that their HPV-1 L1 proteins were of appropriate conformation.

The first property, i.e., molecular weight, merely suggests that whatever HPV-1 L1 sequence cloned by Carter et al was apparently transcribed, translated and the L1 protein was not substantially degraded. However, it is otherwise irrelevant because molecular weight cannot be used to determine whether a protein exhibits a particular conformation.

The second cited property, i.e., the reactivity of the HPV-1 L1 proteins of Carter et al with monoclonal antibodies also does not substantiate a conclusion that these L1 proteins exhibit a conformation characteristic of intact, infectious HPV-1 virions. This is because Carter et al tested the immunoreactivity of their L1 proteins with monoclonal antibodies which bind to linear epitopes. This deficiency of Carter et al is discussed in the most recent Declaration by Dr. Schlegel and Dr. Jenson (dated October 24, 1995) (hereinafter the Schlegel and Jenson §132 Declaration). Therein, the Declarants further note that L1

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proteins which exhibit appropriate conformation (conformation characteristic of native, intact HPV-1 L1 proteins) can only be assessed based on their reactivity with conformationally-dependent antibodies. [*See* paragraph 7 of Schlegel and Jenson §132 Declaration.] Thus, contrary to the Office Action, Carter et al contains no evidence which would allow one skilled in the art to reasonably conclude that their HPV-1 L1 proteins expressed intracellularly in yeast exhibit appropriate conformation.

Moreover, Applicants respectfully maintain that there is sufficient evidence of record to establish that Carter et al most probably did not obtain HPV-1 L1 proteins having appropriate conformation. In particular, the fact that Carter et al. cloned their HPV-1 L1 sequence by polymerase chain reaction (PCR), a cloning technique which is well known to introduce mutation(s), (on average this technique introduces 3-5 nucleotide errors for a nucleotide sequence which is about 1500 nucleotides) substantiates Applicants' position that the HPV-1 L1 sequence expressed by Carter et al most likely did not possess appropriate conformation, i.e., the conformation of native HPV-1 L1 proteins.

This argument finds factual support in the same §132 Declaration of Dr. Schlegel and Dr. Jenson. Notwithstanding this Declaration evidence, the §102(b)/§103 inherency rejection based on Carter et al has been maintained. Essentially, the Examiner reasons that while PCR typically introduces mutations, and that it would be therefore reasonable to assume that the L1 sequence cloned by Carter et al likely contained one or more mutations, that there is no reason to conclude that any of these mutations

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would have affected conformation. The Examiner notes that such mutations could have been silent (not affect amino acid sequence), or alternatively, even if they altered the primary amino acid sequence, may not cause significant affects on the conformation of the resultant HPV-1 L1 protein.

However, this conclusion is respectfully traversed. While Applicants acknowledge that some mutations introduced into the HPV-1 LI sequence would reasonably be expected to exert no effect on conformation of the resultant expressed protein, given the inherent sensitivity of HPV-1 L1 to conformational changes (as evidenced by the drastic effects of a single amino acid change in the HPV-16 L1 sequence), it would be reasonable to conclude that at least one of the expected 3 to 5 mutations in the L1 sequence expressed by Carter et al would have altered the conformation of the resultant HPV-1 L1 protein.

Applicants respectfully note that the initial burden to substantiate an inherency based §102 or §103 rejection is on the Patent Office. (*See*, In re Piasecki, 223 U.S.P.Q. 785 (CAFC 1984).) Moreover, inherency must be certain. Ex parte Cyba, 158 U.S.P.Q. 756 (POBA 1966). Essentially, it must be a necessary result and not merely a possible result. Ex parte Keith et al, 154 U.S.P.Q. 320 (POBA 1966). Only after this initial burden has been met, does the burden then shift to Applicant to rebut the inherency based rejection. In re Murch, 175 U.S.P.Q. 89 (CCPA 1972).

However, based on the evidence of record, Applicants respectfully submit that a proper inherency based rejection has not

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been set forth, essentially because there is no evidence which would allow one skilled in the art to reasonably conclude that the HPV-1 L1 proteins expressed by Carter et al reproduced the antigenicity and conformation of an HPV-1 L1 protein expressed by intact, infectious HPV-1 virions.

Moreover, there is yet even further reason to reasonably conclude that the HPV-1 L1 proteins expressed by Carter et al. would not have exhibited proper conformation. In particular, Applicants note that the HPV-1 L1 proteins of Carter et al were expressed in intracellular form in *Saccharomyces cerevisiae* and then isolated by pelleting the yeast, washing, and disruption by mechanical lysis using glass beads in a buffer containing Tris, EDTA, sodium dodecyl sulfate (SDS), and Triton X-100. (*See* left-hand column, page 515 of Carter et al, lines 4-8.) However, it is well known that SDS is a strong denaturant. In fact, SDS is conventionally used to provide for unfolding of proteins. (*See* page 174 of *Mol. Biol. of the Cell*, Alberts et al, e.d. 1983, attached to this Reply.) Therefore, given the usage of SDS during HPV-1 L1 protein recovery, it is reasonable to conclude that any HPV-1 L1 proteins which were contained in the yeast lysate material disclosed by Carter et al would not exhibit the same conformation as an L1 major capsid protein expressed on the surface of intact, infectious human papillomavirus virions. Rather, it would be expected that one or more conformational epitopes would not be expressed because of the known denaturing effects of SDS.

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Moreover, the §102/103 inherency rejection is separately argued as it is applied to Claims 12 and 15. These claims are directed to a vaccine suitable for the prevention of human papillomavirus infection, which comprises at least one recombinantly produced human papillomavirus virions (PV) L1 protein, which protein reproduces the antigenicity and exhibits the same conformation as an L1 major capsid protein expressed on the surface of intact, infectious human papillomavirus virions.

There is absolutely no basis for concluding that Carter et al teaches or suggests a composition containing HPV-1 L1 proteins which would be suitable for use as a vaccine for prevention of papillomavirus infection. In fact, the reference contains no teaching or suggestion concerning the use of their disclosed HPV-1 L1 proteins, or any of their expressed HPV proteins, for use in vaccines. Rather, the only prophetic usages of the disclosed HPV proteins are as tools for examining humoral immunity to papillomaviruses, as tools for the study of cellular immunity and for structure/function studies of the proteins themselves. (*See*, page 520, last paragraph, preceding "Acknowledgements", of Carter et al.) Therefore, based on the foregoing, withdrawal of the §102(b)/103 rejection of Claims 1-3, 10, 12, 15 and 59-62 based on Carter et al. is respectfully requested.

Claims 13, 14, 18, 19, 21, 25, 46, 47, 50, 51, 53, 54, and 59-62 further stand rejected under 35 U.S.C. §103 as being unpatentable over Carter et al. as applied to Claims 1-3, 10, 12, 15, and 59-62 and further in view of Danos et al (U. S. Patent No. 4,551,270). Essentially, the Examiner asserts that Carter et al

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discloses an HPV-1 L1 protein expressed in yeast which, absent evidence to the contrary, would appear to exhibit the antigenicity and conformation of HPV-1 L1 proteins expressed by intact, native HPV-1 virions, and that in view of Danos et al it would have been obvious to have used such proteins as a vaccine for conferring immunity against HPV-1 infection. This rejection is not made against the claims which do not encompass the use of HPV-1 L1 protein as a vaccine.

For the reasons set forth above, Carter et al does not teach or suggest an HPV-1 L1 protein which would exhibit the antigenicity and conformation of L1 proteins expressed on the surface of intact, infectious HPV-1 virions. Nor does the reference teach or suggest the use of their HPV-1 L1 proteins as vaccines. Rather, the reference teaches the expression of HPV proteins which are assertedly potentially useful for diagnostic use.

The addition of Danos et al does not compensate for the deficiencies of Carter et al. As acknowledged by the Examiner, this reference teaches linear peptides which purportedly are useful in the preparation of HPV vaccines. However, as previously argued, these peptides wold be unsuitable for use in vaccines because linear peptides do not confer immunity against HPV infection. Rather, proper conformation is absolutely <u>essential</u> for a protective immune response. The Examiner indicates that Applicants have failed to indicate wherein the record or in the art it had been previously established that linear HPV-1 proteins and peptides are not useful as vaccines. Also, the Office Action

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indicates that this argument is unpersuasive because issued patents are presumed valid.

However, Applicants respectfully note that the as-filed application specifically discloses that linear molecules corresponding to the L1 protein are incapable of protecting against papillomavirus infection, whereas conformationally correct L1 proteins (produced by the subject invention) are capable of inducing neutralizing antibodies which effectively protect against papillomavirus infection. (*See*, e.g., page 8, lines 8-13, of the as-filed specification.) Indeed, this is the whole crux of the subject invention. With respect to HPV-1 in particular, the application specifically teaches expression of HPV-1 L1 proteins in Cos cells and also contains evidence which demonstrates that the resultant recombinant HPV-1 L1 proteins specifically bind to monoclonal antibodies that recognize conformational epitopes expressed on the surface of HPV-1 particles.

The fact that appropriate conformation is essential for an effective HPV vaccine, i.e. one that protects hosts against infection by the corresponding human papillomavirus virus, is further substantiated by the §132 Declaration dated June 10, 1994, by Dr. Schlegel. This Declaration contains experiments which provide conclusive *in vivo* evidence that conformationally correct papillomavirus proteins confer immunity against papillomavirus infection in susceptible animals. These experiments utilize the COPV/canine animal model. As previously established, COPV is an accepted *in vivo* model for HPV.

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In particular, the results described at pages 13-14 of the Declaration, provide conclusive evidence substantiating the importance, i.e., essentiality, that an L1 protein must appropriate conformation to confer protective immunity, and further that linear epitopes are apparently not involved in conferring protection against PV infection. Specifically, these results indicate that Beagle dogs which were inoculated with recombinant conformationally correct COPV L1 produced a substantial antibody response against COPV conformational epitopes. By contrast, the control group (which were not administered these proteins) exhibited virtually no change in the antibody response to conformational epitopes after challenge.

Moreover, while vaccinated animals developed an immune response to conformational L1 proteins, they failed to develop a significant response to linear, i.e. non-conformational epitopes. This may be appreciated upon review of the results contained in Figure 3 of the Schlegel Declaration. Therefore, these results demonstrate that antibodies to linear epitopes (such as would be obtained upon administration of the HPV-1 L1 proteins of Danos et al) would not confer protection. Based on the fact that COPV is an acceptable model for HPV, it is reasonable to conclude, based on these results, that linear peptides derived from HPV-L1 could not be used to confer protection against HPV-1 infection. More importantly, these results demonstrate that linear and conformational HPV L1 sequences do not function equivalently as immunogens.

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Moreover, there are numerous references of record which substantiate the overwhelming acceptance by those skilled in the art that conformational L1 epitopes are essential for eliciting a protective immune response against papillomavirus virus infection. *See*, e.g., *Gynecological Oncology*, 55:10-12 (1994), entitled "Recombinant Virus-Like Particles Retain Conformational Epitopes of Native Human Papillomaviruses and May be Useful for Vaccine Development"). *See* also, Heinz et al, *Gynecological Oncology*, 55:13-20, (1994), entitled "Role of Conformational Epitopes Expressed by Human Papillomavirus Major Capsid Proteins in the Serologic Detection of Infection and Prophelatic Vaccination".

Therefore, contrary to the Office Action, Applicants respectfully submit that there is substantial evidence of record which demonstrates the importance, i.e. essentiality, of conformational epitopes for eliciting protection against papillomavirus virus infection. Therefore, based on the foregoing, withdrawal of the §103 rejection of Claims 13, 14, 18, 19, 21, 25, 46, 47, 50, 51, 53, 54 and 59-62 under 35 U.S.C. §103 based on Carter et al taken in view of Danos et al is respectfully requested.

Claims 10, 11, 15, 17, 18, 21, 22, 26, 51, 54 and 63 stand newly rejected under 35 U.S.C. §112, first paragraph, as being non-enabled. Essentially, the Office Action asserts that the specification fails to enable synthesis of an HPV-16 L1 protein which reproduces the antigenicity and conformation of L1 proteins expressed by native, intact HPV-16 virions. The Examiner indicates that these claims are not enabled because the HPV-16 L1

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sequence which was publicly known contained a mutation which upon expression resulted in a HPV-16 L1 protein which does not exhibit proper conformation, i.e. does not reproduce the antigenicity and conformation of a HPV-16 L1 protein expressed on the surface of intact, infectious HPV-16 virions. This rejection is respectfully traversed.

While it is acknowledged that the HPV-16 L1 sequence which had been widely reported (HPV-16 L1 sequence of Zur Hausen) contained a mutation which affected conformation, this fact does not substantiate the enablement rejection. Once it had been established that HPV sequences could be expressed having appropriate conformation (as disclosed in the subject application), it would have been well within the level of ordinary skill to clone an HPV-L1 sequence from any infectious human papillomavirus including HPV-16 and to express such L1 sequence according to the teachings of the application in order to obtain the corresponding conformational HPV L1 protein.

Moreover, with particular respect to HPV-16, it had been well known in the art at the time of invention that infectious HPV-16 strains are frequently detected in cervical carcinomas/cervical warts, premalignant cervical lesions and invasive cervical carcinoma. (*See*, Bubb et al, *Virology*, 163:243-246 (1988), attached to this Reply). In fact, this reference exploits this fact by isolating an infectious form of HPV-16 from extra chromosomal viral DNA contained within a premalignant cervical lesion.

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Therefore, based on what had been known in the art at the time of invention, one skilled in the art, in possession of this application, could, absent undue experimentation, clone an infectious form of HPV-16 (e.g., from premalignant cervical tumor), derive the HPV-16 L1 sequence therefrom, and express the L1 sequence using the teachings of this application in order to produce a conformational HPV-16 L1 protein. Moreover, whether the resultant protein exhibits appropriate conformation could readily be determined based on reactivity of the HPV-16 L1 protein with conformational antibodies specific to HPV-16 L1 proteins.

Also, using an infectious HPV-16 rather than an HPV-16 integrant, would have been obvious based on Bubb et al who disclose a HPV-16 DNA integrant which contains a mutation (extra nucleotide) which affects the reading frame of a viral protein (E5 protein). By contrast, the HPV-16 E5 sequence cloned by Bubb et al from pre-malignant cervical lesions lacks such mutation. Therefore, it would have been obvious to have cloned an infectious form of HPV-16 so as to enhance the likelihood that the obtained L1 sequence would not contain mutations which may affect conformation.

Therefore, Applicants respectfully submit that based on the teachings in this application those skilled in the art could practice the invention as it pertains to HPV-16 L1 sequences. Essentially, this would involve cloning an L1 sequence from an infectious strain of HPV-16, expressing such sequence according to the teachings of the application, and assaying whether this

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protein exhibits appropriate conformation (using conformational antibodies). Therefore, withdrawal of the enablement rejection of Claims 10, 11, 15, 17, 18, 21, 22, 26, 51, 54 and 63 is respectfully requested.

Claims 1-3, 10-19, 21-26, 46, 47 and 50-63 further stand newly rejected under 35 U.S.C. §112, first paragraph. The Office Action asserts that the as-filed specification fails to provide support for expression of an L1 protein that reproduces the antigenicity of intact, native human papillomavirus.

By the present amendment, the phrase "intact, native human papillomavirus" has been amended to --intact, infectious human papillomavirus--. As noted above, this language finds explicit support from page 10, lines 1-2 of the application. Therefore, withdrawal of the §112, first paragraph, rejection of Claims 1-3, 10-19, 21-26, 46, 47 and 50-63 is respectfully requested.

Finally, Claims 1-3, 10-19, 21-26, 46, 47 and 50-63 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite. These claims are asserted to be indefinite in the recitation "native" and "mature". By the present amendments, "intact, mature human papillomavirus" has been amended to --intact, infectious human papillomavirus--. Thus, the §112, second paragraph, rejection should now be moot, because the meaning of an infectious form of HPV would be readily apparent to one of ordinary skill in the art. Withdrawal of the §112, second paragraph, rejection of Claims 1-3, 10-19, 21-26, 46, 47 and 50-63 is therefore respectfully requested.

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Based on the foregoing, this application is believed to be in condition for allowance. A Notice to that effect is respectfully solicited. However, if any issues remain outstanding, the Examiner is respectfully requested to contact the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,

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